July 28, 1970

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Dear Dr. Appleyard:

When EMBO awarded me the fellowship to go to Michael Stoker's laboratory in London, you indicated that a final report on the trip was necessary. I should like, in this letter, therefore, to provide you with that summary.

But, before I do so, let me say again how much I appreciated the support which EMBO provided and how useful it was to accomplish the project we set out to do. Not only was the stay in London productive and pleasant, but I must say that dealing with the EMBO organization proved to be a model of efficiency.

In the following statement, I have summarized the purposes for which I requested EMBO support, what was accomplished during the fellowship tenure, and what has yet to be done to complete the project. In addition, I report on the expenditure of the funds which were advanced to me under your fellowship grant.

A. Purpose of Visit to Imperial Cancer Research Fund Laboratory:

Most people accept the view that oncogenic transformation of certain mammalian cells by the DNA-viruses, polyoma (PY) and SV40, is accompanied by integration of the viral genome into the host's chromosomal complement: In most cases multiple copies of the viral DNA sequences appear to be covalently linked to the cellular DNA; but whether the viral DNA copies are clustered in one location or whether they are distributed throughout the host's chromosomal DNA is not yet certain.

Several years ago Michael Stoker, now at the ICRF laboratory in London, showed that BHK cells (a line of cells which fails to multiply polyoma virus), became "transiently transformed" when infected with high input multiplicities of polyoma virus: In this case "transient transformation" refers to the temporary acquisition of the ability of the infected cells to divide and form small clones in Methocel, a property which is common to the transformed cell but

which is not shared by normal uninfected cells. Most importantly, however, is the fact that although a majority of the cells become "transiently transformed" (>70%), most of these revert to the morphology and growth characteristics of the parental non-transformed cell; only a small fraction (<10%) of the cells acquire a stable transformed phenotype. Stoker refers to this phenomenon as "Abortive Transformation".

A pertinent question which I want to answer is: Does the transient transformatinn result from expression of the infecting viral genome and does the consequent reversion occur by elimination of the viral genetic information from the cell? Or does reversion occur because integration of the viral genome occurs in such a way as to turn off or "repress" viral gene expression and thereby restore normal cellular control over growth? Quite conceivably one might argue that integration of viral genes into the host cell genome is essential but not sufficient to cause stable transformation; what is essential is that the integrated viral genes continue to be expressed.

Accordingly, Stoker and I set out to examine stable and transiently or abortively transformed cells for their content of viral DNA sequences. The first step was to isolate from the same infected culture a collection of clones grown from stable and abortively transformed cells. I was then to take these clones back to my laboratory at Stanford to determine, by an improved hybridization procedure (see Westphal and Dulbecco, PNAS 59:1158 (1968)), whether the cells contain DNA sequences homologous to the viral DNA used in the infection.

B. Accomplishments

During the period in which I was at the ICRF laboratory (June 12-29) I was able, with the collaboration of Dr. Stoker and his colleagues to carry out two different types of experiments for generating abortive and stable transformants of BHK following infection with PY (1 and 2). Our attempts to induce "abortive transformation" or more correctly induction of cellular DNA synthesis in 3T3 following SV40 infection were not successful although further experiments are in progress in my home laboratory (3).

1) In this experiment BHK cells were infected with PY and then seeded into medium containing Methocel. Clones of various sizes were picked from the Methocel after about a fortnight and seeded in liquid medium. After several days of growth they were scored as transformed and normal colony morphology. Several

of each type were picked and replated to obtain pure colonies of normal and transformed cells. Since only clones which had been stimulated to multiply by the infection were picked from the Methocel, we presumably have a sampling of the progeny of abortive and stable transformants of the type we wanted.

2) In this experiment we used induction of cellular DNA synthesis as an indicator of abortive transformation. BHK cells which had been limited in their growth (and DNA synthesis) by being kept in a medium deficient in an essential serum factor(s) were infected with PY and then exposed to ^{3}H-TdR in the deficient medium. In infected cultures 65-90% of the cells showed radioactive nuclei (by radioautography) while uninfected cultures contained of the order of 1% labeled nuclei. Such "stimulated" cells were seeded in normal medium to produce clones and from these plates I selected, at random, about ten normal growing colonies and about 5 transformed colonies. Each was passaged to produce pure cultures of the respective normal (many of these, however, were presumably derived from the stimulated cells) and stably transformed cells.

3) 3T3 cells arrested in G1 phase by serum factor starvation showed little or no stimulation of DNA synthesis over that of mock-infected cells. We could also not detect any dramatic synthesis of SV40 T-antigen in the infected cells. The reasons for this failure are not yet known but I am trying now, in my own laboratory, to find the appropriate conditions for inducing abortive transformation in this system.

4) A more general accomplishment of my stay at ICRF were the many opportunities for discussion with Dr. Stoker and his colleagues about their's and our experimental approaches to the problem of viral oncogenesis. These were exciting and certainly helped my thinking about the field. In addition I picked up several techniques we can now incorporate into our own work (autoradiography, detection of T-antigen by fluorescent staining and general tissue culture techniques).

C) What has to be done?

We are currently accumulating the reagents (viral DNA, labeled RNA) and the technique of the hybridization (primarily trying to lower the background) to examine the clones we produced for the presence or absence of the viral DNA.

D) Report on Expenditures

I was advanced $956 for the trip: $577 was for travel to and from London and the remainder to cover expenses while living in London. In connection with my participation as a speaker at a Royal Society Discussion Meeting on Viruses as Genetic Modifiers of Cells, I was given $350 towards my travel expenses by the Royal Society; therefore I am enclosing my personal check in that amount for return to EMBO. The remainder was used to help pay hotel, living and other related costs associated with my travel to and from England and my stay there.

Sincerely,

Paul Berg

PB/i

enclosure